Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Manganese Sulfate** in the developing chicken embryo 2/11/76

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MORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

: GRAS Review Branch, HFF-335

DATE: February 11, 1976

Manganese

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: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Manganese Sulfate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Manganese Sulfate

Protocol:

Manganese Sulfate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Air cell administration of Manganese Sulfate resulted in an LD_{50} of 162.088 mg/kg (8.104 mg/egg) at 0 hours and an LD_{50} of 3.650 mg/kg (0.1825 mg/egg) at 96 hours. No LD_{50} could be determined with yolk administration, since at 0 hours the line had a negative slope, and at 96 hours the slope of the line was not significantly different from zero ($p=0.05$).

Scattered minor abnormalities were observed for the four test conditions but in no instance were they different from or significantly higher than those observed in the background. Manganese Sulfate displayed no teratogenicity under the test conditions employed.

1. Manganese Sulfate, Lot # ZGT, Mallinckrodt Chem. Works, St. Louis, Mo.

2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Manganese Sulfate
Air Cell @ 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	70	70.00*	1.42	0.00
5.00	100.00	70	31.42*	4.28	0.00
2.50	50.00	99	18.18	1.01	0.00
1.250	25.00	120	20.83	1.66	0.83
0.50	10.00	120	23.33	2.50	0.83
Water	--	160	17.50	1.87	1.25
Controls	--	421	8.07	1.90	1.66

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 162.088 mg/kg (8.104 mg/egg)

Manganese Sulfate
Yolk @ 9 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	45	66.66*	0.00	0.00
5.00	100.00	45	71.11*	0.00	0.00
2.50	50.00	70	60.00*	0.00	0.00
1.250	25.00	70	67.14*	1.42	0.00
0.500	10.00	115	66.95*	0.00	0.00
Water	--	130	20.00	0.76	0.76
Controls	--	421	8.07	1.90	1.66

*Significantly different from solvent p_0.05
**Slope is negative

Manganese Sulfate
Air Cell @ 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
0.6250	12.500	70	90.00*	2.85	0.00
0.250	5.000	120	65.83*	2.50	1.66
0.1250	2.500	120	40.00*	0.83	0.00
0.06250	1.250	120	32.50*	0.00	0.00
0.0250	0.500	120	20.00	3.33	1.66
Water	--	150	14.00	1.33	0.00
Controls	--	421	8.07	1.90	1.66

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 3.650 mg/kg (0.1825 mg/egg)

Manganese Sulfate
Yolk @ 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs.	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	90	55.55*	5.55	1.11
2.50	50.00	85	50.58*	2.35	0.00
1.250	25.00	110	46.36*	1.81	0.90
0.6250	12.50	110	52.72*	6.36	4.54
0.250	5.00	110	38.18*	4.54	2.72
Water	--	130	22.30	1.53	1.53
Controls	--	421	8.07	1.90	1.66

*Significantly different form solvent $p \leq 0.05$
**Slope not significantly different from zero $p=0.05$